

551,608

## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
21 October 2004 (21.10.2004)

PCT

(10) International Publication Number  
**WO 2004/090109 A2**

- (51) International Patent Classification<sup>7</sup>: **C12N**
- (21) International Application Number:  
PCT/US2004/009810
- (22) International Filing Date: 31 March 2004 (31.03.2004)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
60/459,361 31 March 2003 (31.03.2003) US
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- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**  
— without international search report and to be republished upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CCN3 COMPOSITIONS AND METHODS

(57) Abstract: CCN3 (Nov) is a matricellular protein of the CCN family, which also includes CCN1 (CYR61), CCN2 (CTGF), CCN4 (WISP-1), CCN5 (WISP-2), and CCN6 (WISP-3). During development, CCN3 is expressed widely in derivatives of all three germ layers, and high levels of expression is observed in smooth muscle cells of the arterial vessel wall. Altered expression of CCN3 has been observed in a variety of tumors, including hepatocellular carcinomas, Wilm's tumors, Ewing's sarcomas, gliomas, rhabdomyosarcomas, and adrenocortical carcinomas. To understand its biological functions, we have investigated the activities of purified recombinant CCN3. We show that in endothelial cells, CCN3 supports cell adhesion, induces directed cell migration (chemotaxis), and promotes cell survival. Mechanistically, CCN3 supports human umbilical vein endothelial cell adhesion through multiple cell surface receptors, including integrins  $\alpha_v\beta_3$ ,  $\alpha_5\beta_1$ ,  $\alpha_6\beta_1$ , and heparan sulfate proteoglycans. In contrast, CCN3-induced cell migration is dependent on integrins  $\alpha_v\beta_3$  and  $\alpha_5\beta_1$ , whereas  $\alpha_6\beta_1$  does not play a role in this process. Although CCN3 does not contain a RGD sequence, it binds directly to immobilized integrins  $\alpha_v\beta_3$  and  $\alpha_5\beta_1$  with half maximal binding occurring at 10 nM and 50 nM CCN3, respectively. Furthermore, CCN3 induces neovascularization when implanted in rat cornea, demonstrating that it is a novel angiogenic inducer. Together, these findings show that CCN3 is a ligand of integrins  $\alpha_v\beta_3$  and  $\alpha_5\beta_1$ , acts directly upon endothelial cells to stimulate pro-angiogenic activities, and induces angiogenesis *in vivo*.

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